ORIGINAL ARTICLE

Frequency of AFB smear and GeneXpert positivity in bronchoalveolar lavage samples of smear-negative suspected pulmonary tuberculosis patients

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ABSTRACT

Background: Pulmonary tuberculosis (TB) patients with negative sputum smears is a daunting task. Bronchoalveolar lavage (BAL) can get lower respiratory samples for analysis. We evaluated the frequency of AFB smear and GeneXpert positivity on BAL samples of smear-negative suspected pulmonary TB.

Material and Methods: This cross-sectional study conducted at the Department of Pulmonology, Ojha Institute of Chest Disease, Karachi, Pakistan from 1st July 2025 to 30th November 2025. Patients aged 20 to 70 years, either gender, suspected of having pulmonary TB and negative for AFB on at least three consecutive sputum smear samples were included. All patients underwent bronchoscopy and BAL. BAL fluid was tested for *Mycobacterium* TB using both AFB smear microscopy and the GeneXpert MTB/RIF assay.

Results: Out of 150 patients, 62 patients had a positive AFB smear on the BAL fluid, yielding a BAL smear positivity rate of 41.3% (95% CI: 33.5%–49.2%). In contrast, 100 patients had *M. TB* detected by GeneXpert MTB/RIF on their BAL fluid, corresponding to a BAL GeneXpert positivity rate of 66.7% (95% CI 59.1%–74.2%). AFB smear positivity was significantly higher among rural residence (n=28, 56%) as compared to urban residence (n=34, 34%) (p=0.010). Whereas, GeneXpert positivity on BAL was higher among unemployed (n=59, 75.6%) as compared to employed (n=41, 56.9%) (p=0.015).

Conclusion: Bronchoscopy with BAL significantly improved the diagnostic yield in sputum smear-negative presumptive TB patients. GeneXpert testing on BAL fluid identified a significantly more TB cases than smear microscopy, thus emphasizing its role for rapid, confirmatory diagnosis.

Keywords: AFB smear, Bronchoalveolar lavage, GeneXpert positivity, Pulmonary tuberculosis, Respiratory disease

BACKGROUND

Tuberculosis (TB) remains one of the most common and deadly infectious diseases worldwide.[1] In 2021, an estimated 10.6 million people developed TB globally and 1.6 million died from the disease.[1,2] Pakistan is ranked among the top five high TB burden countries, accounting for approximately 5–6% of global TB cases.[3] Prompt and accurate diagnosis of pulmonary TB is critical for patient outcomes and for preventing ongoing transmission.[3] However, a substantial proportion of pulmonary TB cases are smear-negative, meaning they have no acid-fast bacilli on sputum microscopy despite active disease.[4] Studies indicate that more than half of pulmonary TB cases can be smear-negative. [4]

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Sputum smear microscopy has a sensitivity of only around 44–60% under programmatic conditions and even lower sensitivity in paucibacillary disease and immunocompromised patients.[5] This diagnostic gap leads to missed or delayed treatment, particularly in patients relying solely on clinical and radiological findings.[5] To address this, international guidelines therefore emphasize the need for further diagnostic tools in smear-negative suspected TB, such as nucleic acid amplification techniques.[6]

In recent years, Xpert MTB/RIF (GeneXpert) a rapid cartridge-based polymerase chain reaction test has transformed the diagnosis of TB by detecting *Mycobacterium tuberculosis* DNA and rifampicin resistance, has significantly improved diagnostic accuracy.[7] With a lower detection threshold than microscopy and sensitivity up to 89% in culture-confirmed TB, GeneXpert is now endorsed by WHO as a frontline diagnostic tool, especially for smear-negative patients.[5,7]

Another valuable tool for diagnosing smear-negative TB is bronchoscopy with bronchoalveolar lavage

(BAL).[4] Flexible fiber-optic bronchoscopy permits sampling of the lower respiratory tract, increasing the chance of obtaining bacilli from deep lung segments not represented in expectorated sputum.[8] BAL fluid can then be examined by smear, culture, and molecular tests. Bronchoscopic sampling is especially useful in patients who cannot produce sputum or when sputum tests are non-diagnostic.[8,9] Prior studies have demonstrated that bronchoscopy can substantially improve diagnostic yield – yielding a microbiological diagnosis in about 30% up to 80–90% of smear-negative TB cases, depending on the combination of tests used.[6,10] Moreover, when GeneXpert is applied to BAL fluid, diagnostic yields increase even further. These data underscore that combining bronchoscopy with rapid molecular testing could close the diagnostic gap for smear-negative TB. [5,7,11]

In a country like Pakistan where burden of TB is high and resources are limited for timely diagnosis, delay in identifying smear-negative TB leads to poor outcomes. Yet, there is scarcity of data on the utility of BAL in smear-negative TB are scarce. Hence, this study assessed the frequency of AFB smear and GeneXpert positivity on BAL samples in sputum smear-negative, suspected pulmonary TB cases at a tertiary hospital in Karachi.

MATERIAL AND METHODS

It was a cross-sectional study conducted at the Department of Pulmonology, Ojha Institute of Chest Disease, Karachi from 1st July 2025 to 30th November 2025. The study's sample size was determined to be 150 patients, calculated using the World Health Organization's (WHO) software, (based on 38% AFB smear positivity[9], 8% margin of error, 95% CI). Patients aged 20 to 70 years, either male or female, clinically and radiologically suspected of having pulmonary TB and negative for AFB on at least three consecutive sputum smear samples were included. Suspected TB was defined as presence of a new radiographic abnormality (opacity with or without air bronchograms) along with at least one of the following symptoms: productive cough, fever (>37.8°C), or weight loss ≥10 kg. Patients with history of lung cancer, HIV, ILD, COVID-19, comorbidities such as asthma, COPD, MI, CHF, CKD, stroke, history of fungal infections (e.g., histoplasmosis, blastomycosis), diagnosed sarcoidosis, and pregnant women were excluded from the study. All eligible patients who fulfilled the inclusion criteria were enrolled using non-probability consecutive sampling technique, and none declined from bronchoscopy or were excluded after recruitment.

This research was conducted at the Ojha Institute of Chest Disease in Karachi, following the approval of the relevant ethics committees. Written informed consent was obtained from all the eligible patients. Data regarding demographic information including age, gender, employment status, family income, residence, and education were recorded.

All patients underwent bronchoscopy performed by experienced pulmonologists under conscious sedation in a dedicated bronchoscopy suite. All patients underwent bronchoscopy performed by experienced pulmonologists under conscious sedation in a dedicated bronchoscopy suite. Pre-procedure, patients were kept fasting and received topical lidocaine and low-dose midazolam as needed for comfort. Continuous monitoring of oxygen saturation, blood pressure, and heart rhythm was maintained throughout. A flexible fiber-optic bronchoscope was inserted transorally or transnasally into the bronchial tree. Bronchoalveolar lavage was performed in the lung segment corresponding to radiographic lesions or, if diffuse infiltrates were present, in the right middle lobe or lingula. Sterile normal saline (up to 100 mL in aliquots of 20–50 mL) was instilled through the bronchoscope and then aspirated to collect the lavage fluid. The BAL fluid return was collected in sterile containers and promptly sent to the laboratory for TB testing. In the laboratory, each BAL sample was divided for two tests. A portion of the BAL fluid was centrifuged and the sediment smeared on slides, stained by Ziehl-Neelsen technique, and examined under light microscopy for acid-fast bacilli. Smears were graded according to standard guidelines (no AFB in 100 fields = negative; 1–9 AFB/100 fields = +1, etc.). For this study, any grade ≥1+ was considered positive for AFB smear. Another portion of BAL fluid (≥1 mL) was processed using the Xpert MTB/RIF cartridge system (Cepheid, USA) as per manufacturer instructions. In brief, sample reagent was added in a 2:1 ratio, the mixture incubated for 15 minutes with intermittent shaking, then 2 mL aliquot was loaded into the cartridge. The automated real-time PCR system detected *M. tuberculosis* DNA and rifampicin resistance mutations. Results were reported as “MTB detected” (with semiquantitative bacillary load and RIF resistance detected or not) or “MTB not detected”. For study purposes, any “MTB detected” result was considered GeneXpert positive for TB.

The primary outcomes were: The frequency of BAL AFB smear positivity (number and percentage of patients with AFB seen on BAL fluid smear) and the frequency of BAL GeneXpert MTB positivity (number and percentage of patients with *M. tuberculosis* detected by Xpert in BAL fluid).

The data analysis was performed using Statistical Packages for Social Sciences (SPSS) software version 23. Descriptive statistics including mean and standard deviation for continuous variable like age was computed (Normality of the data was assessed using Shapiro-Wilks test). Frequency and percentage distributions for categorical variables like gender, residence status, family monthly income, occupational status, educational status, AFB smear positivity, and Gene Xpert MTB test positivity on BAL were reported. Stratified analysis for age, gender, residence status, family monthly income, occupational status, and educational status, was conducted to examine the relationship outcome variables i.e. AFB smear positivity and Gene Xpert MTB test positivity on BAL. Post-stratification, Chi-square test, was be used. A p-value ≤ 0.05 was considered as statistically significant.

RESULTS

A total of 150 patients with sputum smear-negative, presumptive pulmonary TB were included (Figure-I). The demographic profile is summarized in Table-I. The mean age was 46.6±14.7 years. Just over 60% were male (n=91) and and two-thirds of patients (66.7%) resided in urban areas of Karachi. About 78 patients (52.0%) were unemployed, 36 (24.0%) had higher (college/university) education, and 101 patients (67.3%) had a monthly household income ≤ 50,000 Pakistani Rupees.

Out of 150 patients, 62 patients had a positive AFB smear on the BAL fluid, yielding a BAL smear positivity rate of 41.3% (95% CI: 33.5%–49.2%). In contrast, 100 patients had *M. tuberculosis* detected by GeneXpert MTB/RIF on their BAL fluid, corresponding to a BAL GeneXpert positivity rate of 66.7% (95% CI 59.1%–74.2%) (Figure I-II).

When comparing the two tests, GeneXpert detected an additional 61 cases that were missed by BAL smear (i.e. 61 patients had a negative AFB smear but a positive GeneXpert on BAL). On the other hand, 23 patients had a positive AFB smear on BAL and a Negative GeneXpert result. The remaining 27 patients (18.0%) were negative on both BAL smear and GeneXpert. Hence 39 out of 150 (26%) were correctly diagnosed as *MTB* on AFB Smear while 100 out of 150 (66.66) were correctly diagnosed as MTB by GeneXpert.

Residence showed a significant association with BAL AFB smear positivity. Rural residents had a higher positivity rate (n=28, 56.0%) compared to urban residents (n=34, 34.0%). No significant difference in AFB positivity between younger (≤45 years, 41.8%) and older (>45 years, 41.0%) patients (p=0.919). Although females had a higher positivity rate (49.2%) than males (36.3%), the difference was not statistically significant (p = 0.117). Occupation, education, and income also showed no statistically significant associations with AFB smear results (all p>0.05) (Table-II)

Occupation was significantly associated with GeneXpert positivity (p=0.015). Unemployed patients had a markedly higher GeneXpert positivity rate (n=59, 75.6%) compared to employed individuals (n=41, 56.9%). Age, gender, residence, education, and income did not show significant differences in GeneXpert positivity (all p>0.05) (Table-III).

Table-I: Baseline characteristics of the patients (n=150).

Variable	Value
Age (years)	46.6 ± 14.7*
Gender	
Male	91(60.7%)
Female	59(39.3%)
Residence	
Urban	100(66.7%)
Rural	50(33.3%)
Occupation	
Employed	72(48.0%)
Unemployed	78(52.0%)
Education	
Illiterate	39(26.0%)
Primary	36(24.0%)

Secondary	39(26.0%)
Higher	36(24.0%)
Income	
≤50,000 PKR	101(67.3%)
>50,000 PKR	49(32.7%)

*Data presented as mean±SD or n (%), *Shapiro-Wilk test was applied, p>0.05.*

Table-II: Association of AFB smear positivity on BAL by demographic variables (n=150).

Variable	Category	AFB Negative (n= 88)	AFB Positive (n=62)	p-value
Age group	≤45 years	39 (58.2%)	28 (41.8%)	0.919
	>45 years	49 (59.0%)	34 (41.0%)	
Gender	Male	58 (63.7%)	33 (36.3%)	0.117
	Female	30 (50.8%)	29 (49.2%)	
Residence	Urban	66 (66.0%)	34 (34.0%)	0.010*
	Rural	22 (44.0%)	28 (56.0%)	
Occupation	Employed	40 (55.6%)	32 (44.4%)	0.457
	Unemployed	48 (61.5%)	30 (38.5%)	
Education	Illiterate	23 (59.0%)	16 (41.0%)	0.493
	Primary	23 (63.9%)	13 (36.1%)	
	Secondary	19 (48.7%)	20 (51.3%)	
	Higher	23 (63.9%)	13 (36.1%)	
Income	≤50,000 PKR	60 (59.4%)	41 (40.6%)	0.792
	>50,000 PKR	28 (57.1%)	21 (42.9%)	

**Significant at 5% level of significance*

Table-III: Association of GeneXpert positivity on BAL by demographic variables (n=150).

Variable	Category	GeneXpert Negative (n=50)	GeneXpert Positive (n=100)	p-value
Age group	≤45 years	24 (35.8%)	43 (64.2%)	0.561
	>45 years	26 (31.3%)	57 (68.7%)	
Gender	Male	29 (31.9%)	62 (68.1%)	0.636
	Female	21 (35.6%)	38 (64.4%)	
Residence	Urban	29 (29.0%)	71 (71.0%)	0.111
	Rural	21 (42.0%)	29 (58.0%)	
Occupation	Employed	31 (43.1%)	41 (56.9%)	0.015*
	Unemployed	19 (24.4%)	59 (75.6%)	
Education	Illiterate	12 (30.8%)	27 (69.2%)	0.866
	Primary	14 (38.9%)	22 (61.1%)	
	Secondary	12 (30.8%)	27 (69.2%)	
	Higher	12 (33.3%)	24 (66.7%)	
Income	≤50,000 PKR	31 (30.7%)	70 (69.3%)	0.325
	>50,000 PKR	19 (38.8%)	30 (61.2%)	

*Significant at 5% level of significance

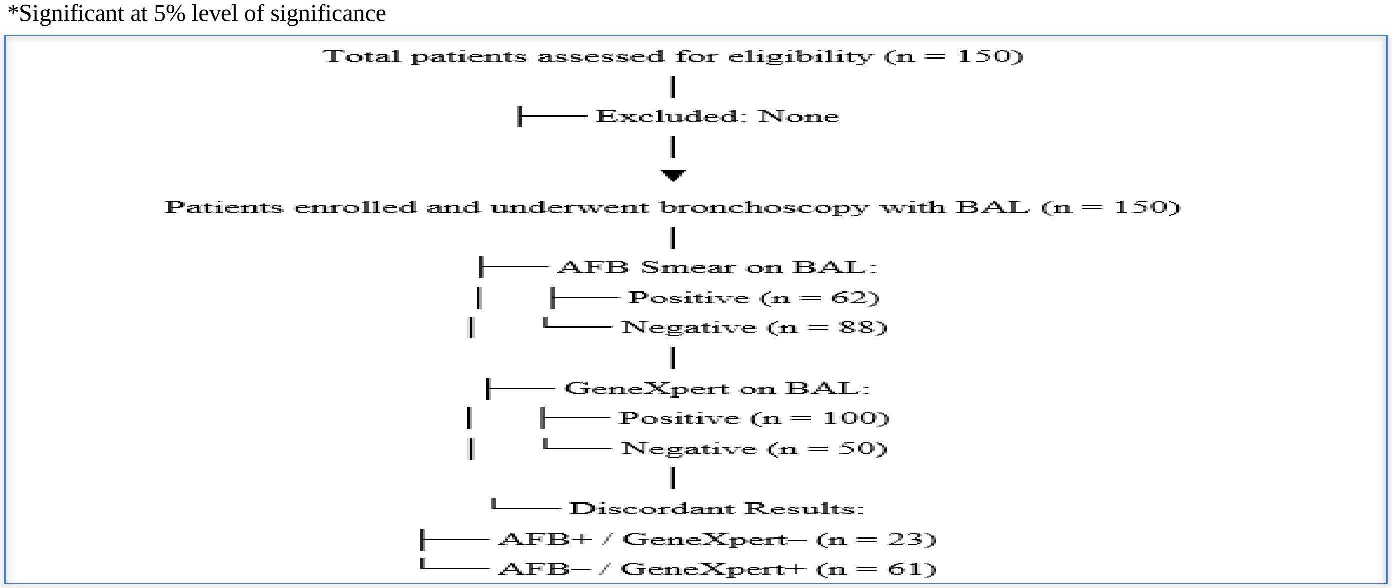

Figure-I: Flow diagram.

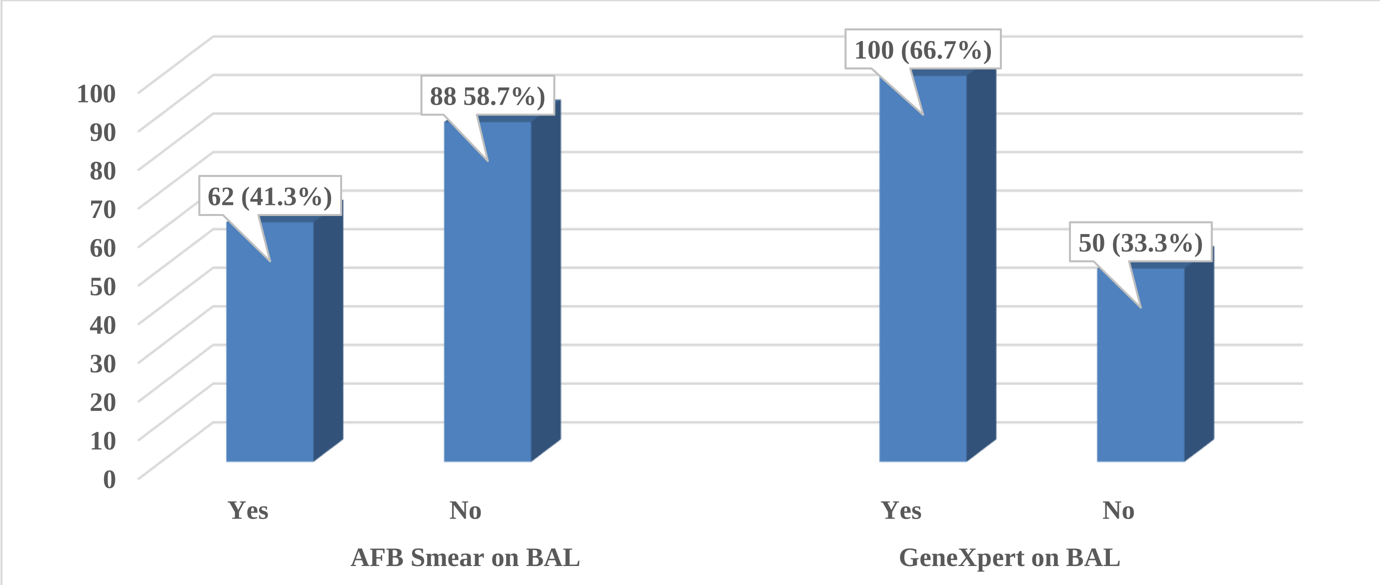

Figure-II: Frequency distribution of AFB smear & GeneXpert on BAL (n=150).

DISCUSSION

We evaluated in this study the utility of bronchoalveolar lavage testing in the diagnosis of pulmonary tuberculosis among patients with negative sputum smears. Our findings indicate that BAL is a very useful diagnostic modality in this setting, especially when modern molecular methods are applied. In smear-negative, suspected TB patients, we report a 41.3% BAL AFB smear positivity and 66.7% BAL GeneXpert MTB/ RIF positivity. These point to the fact that a considerable part of smear-negative TB cases can indeed be confirmed microbiologically by invasive sampling and sensitive testing. Such cases require early confirmation of TB diagnosis, as this enables the timely initiation of treatment and reduces the duration of ongoing transmission.[9, 12]

Our BAL smear positivity rate of 41% was within the range reported in other studies. Nikbakhsh *et al.* in Iran reported a 38% AFB smear-positive rate on BAL in sputum smear-negative TB suspects.[9] Similarly, studies from other high-burden settings have found that roughly one-third to half of smear-negative patients can yield AFB-positive BAL samples.[9,13,14] While BAL smear microscopy improves upon sputum-based smear by accessing deeper pulmonary secretions, its sensitivity remains limited. On the other hand, the GeneXpert MTB/ RIF assay substantially improved in two-thirds of our patients. GeneXpert's 67% positivity in BAL is slightly lower than some reports. Khalil *et al*. reported GeneXpert positivity up to 87% on BAL.[15] The lower yield in our study could be due to differences in patient selection or disease severity. Khalil's study included both smear-negative and sputum-scarce patients and was conducted soon after GeneXpert's introduction, possibly with most patients being highly suspected of TB (some might have higher bacillary load).[15] Our cohort included a mix of patients, some of whom had milder disease or even had already tested GeneXpert-negative on sputum, which could lower the observed positivity rate on BAL. Nonetheless, our results clearly demonstrate the advantage of GeneXpert over smear: it detected an additional 61 TB cases that would have been missed if only smear microscopy was used on BAL.

Interestingly, we observed discordant results in a number of cases: 23 patients were BAL smear-positive but GeneXpert-negative, which are morphologically indistinguishable from *Mycobacterium tuberculosis* on smear but not detected by the GeneXpert assay, which targets TB-specific DNA sequences. Although local NTM prevalence data in Pakistan are limited, NTM has been increasingly recognized in patients with structural lung disease or environmental exposure. Unfortunately, culture and speciation were not performed, representing a study limitation.

One possible explanation is the presence of non-tuberculous mycobacteria (NTM) in those cases. Smear microscopy cannot distinguish NTM from *M. tuberculosis*, whereas the Xpert assay is specific for *M. tuberculosis* complex DNA. It is known that patients with chronic lung conditions or environmental mycobacteria exposure can have AFB-positive smears with negative Xpert/PCR and culture for *M. tuberculosis.*[16] Performing Xpert testing in smear-positive cases therefore has the practical advantage of identifying such discordant results, which may raise suspicion for NTM infection. This can prevent misdiagnosis and unnecessary TB treatment while prompting further confirmatory testing. Previous studies and guidelines have highlighted that smear-positive/Xpert-negative results may serve as an early indicator of NTM, particularly in high-burden settings where smear microscopy is widely used.[22,23] Another explanation could be technical issues or lower sensitivity of Xpert in those particular cases; while GeneXpert is very sensitive, it is not infallible. Xpert's sensitivity in smear-positive samples is reported to be >95%, but false negatives can occur due to PCR inhibitors in the specimen or uneven distribution of organisms. Alternative explanations for GeneXpert false negatives include PCR inhibitors, low bacillary load, or sample degradation. Despite these uncertainties, such discordant cases comprised a minority (15%) of our positive results, and the overall diagnostic yield combining both tests was 82%, supporting their complementary use. Jacomelli *et al*. found that bronchoscopy with BAL had about 60% sensitivity for TB diagnosis on its own, but adding post-bronchoscopy sputum culture and transbronchial biopsy increased the diagnostic sensitivity further.[17] Similarly, other authors have noted that combining multiple bronchoscopic techniques can raise sensitivity into the 80–90% range.[9,18] Our approach effectively combined smear and Xpert, which likely approaches the sensitivity of smear plus culture (GeneXpert has approximately 80%–90% sensitivity compared to culture in smear-negative TB).

It is important to highlight the clinical implications of these findings in a high TB burden country like Pakistan.[19] Sputum smear-negative TB patients pose a diagnostic dilemma: if left undiagnosed, they continue to suffer and can still transmit TB, albeit less efficiently than smear-positive cases.[20] On the other hand, treating patients empirically without confirmation can lead to over-treatment or missing alternative diagnoses, and the indiscriminate use of TB therapy contributes to drug resistance development.[21] In this study, we have shown that bronchoscopy with BAL, along with GeneXpert testing, is a highly effective approach to confirm TB in the majority of smear-negative suspects. This means that many such patients who otherwise would be left unconfirmed (and possibly remain untreated or are empirically treated) can actually be given a definite diagnosis. Early confirmation allows for a prompt institution of appropriate therapy, which leads to an improvement in the outcomes of the patients and interrupts the chain of transmission.[12]

We noted two interesting associations from our data: rural residence was associated with higher BAL smear positivity, while unemployment was associated with higher BAL GeneXpert positivity. Patients from rural areas had a 56% BAL smear positivity compared to 34% in urban patients. This may be because rural patients experience longer delays in seeking care due to access issues, leading to more advanced disease by the time of bronchoscopy (and thus higher mycobacterial load in the lungs, detectable by smear). Urban patients might have received earlier interventions or partially treated disease that lowered bacillary counts. Similarly, unemployed patients-who likely have lower income and less access to healthcare-might present later or have risk factors (poor nutrition, crowded living conditions) that facilitate more extensive disease, resulting in a higher proportion being GeneXpert-positive. These sociodemographic disparities underscore the need for equitable access to diagnostic services. They also suggest that patients of lower socioeconomic means or from rural areas could benefit from earlier referral for bronchoscopy when TB is suspected, to avoid diagnostic delay. That said, these findings should be interpreted with caution due to the sample size and multiple comparisons; further research could explore these trends in a larger population.

Our study has some limitations. First, we did not perform a mycobacterial culture of BAL specimens, which is the gold standard for TB diagnosis. While culture was beyond the scope of our resource setting, its absence means we cannot calculate the true sensitivity of BAL smear or GeneXpert against a definitive reference. As a result, some cases classified as "negative" might actually have had TB that both smear and Xpert missed (false negatives). Prior studies have shown that adding BAL culture can yield an additional 10–20% diagnoses beyond smear and Xpert.[9,16] Second, we did not perform transbronchial lung biopsy or bronchial brushings, which could have further increased diagnostic yield, particularly in patients with atypical presentations or differential diagnoses. Third, this was a single-center study conducted at a tertiary referral hospital. Our population likely had a high pre-test probability of TB, as all patients were evaluated by pulmonologists and selected for bronchoscopy, introducing possible spectrum bias. Results may not be generalizable to primary care or community settings with lower TB suspicion. Additionally, clinical outcomes of patients (e.g., response to treatment, follow-up diagnostics) were not systematically captured, limiting assessment of the impact of diagnostic confirmation.

Despite these limitations, our findings have practical implications. We provide quantitative evidence supporting the use of bronchoscopy with BAL and GeneXpert in patients with smear-negative suspected TB. The combination offers high diagnostic yield, confirms a large fraction of clinically suspected cases, and enables faster treatment decisions. These results may guide pulmonologists, infectious disease specialists, and TB program managers in optimizing diagnostic strategies for smear-negative TB—an often overlooked but clinically significant subset of TB burden.

CONCLUSION

In sputum smear-negative presumptive TB patients, bronchoscopy with BAL substantially improved diagnostic yield. GeneXpert testing on BAL fluid detected a significantly higher proportion of TB cases than smear microscopy alone, highlighting its value for rapid, confirmatory diagnosis. Our findings support the routine use of BAL GeneXpert in smear-negative TB suspects to enable earlier diagnosis and treatment, particularly in high TB burden settings like Pakistan.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Rabeea Nouman: Acquisition, analysis, and interpretation of data, manuscript writing, critical revisions, final approval, accountable for all aspects of publication

Faisal Asad: Critical revisions, final approval, accountable for all aspects of publication

Aisha Asim: Acquisition and analysis, of data, final approval, accountable for all aspects of publication

Nida Shaikh: Manuscript writing, critical revisions, final approval, accountable for all aspects of publication.

Aftab Ahmed: Study conception, manuscript writing, critical revisions, final approval, accountable for all aspects of publication.

Wajeeha Kirmani: Interpretation of data, critical revisions, final approval, accountable for all aspects of publication

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